Effective diffusion coefficients measurement in polysaccharide based hydrogels.

**Aim of the work**

To estimate effective diffusion coefficients of substrate diffusion from limited volume of solution into spherical particles by sorption method.

**Theoretical introduction**

Obtaining of effective diffusion coefficients has high value for modeling and design of bioreactors with immobilized cells. Effective diffusion coefficient is integral parameter which is quantitative characteristic of difficult physical or physico-chemical process. The only possibility of obtaining reliable values of effective diffusion coefficients is experiment. Immobilized enzyme (biocatalyst) is physically bounded or localized in defined area while its enzymatic activity remains, so can be used periodically. In present the most used immobilization technique is entrapment of biocatalyst into hydrophilic matrix of such polymerization level that leads to achieving of pores with desirable size which do not allow high molecular enzymes or cells freely diffuse from carrier unlike substrates and products of reaction. Except synthetic polymers (e.g. polyacrylamide) are also native polymers of polysaccharide nature used (alginate, agar, pectate, carageenane and others), which are in present the most used materials for cells immobilization. Despite the fact that there are some differences in their structure, techniques of their preparation and also characteristic, they are in many aspects very alike, especially by mean of chemical engineering.

Calcium alginate is normally prepared in form of spherical particles by dropping of dissolved calcium alginate to solution of calcium chloride. Bivalent cation diffuses from outer solution into a particle, where cross linking take place. This reaction is very fast so gel particles remains in spherical shape. The reaction take place in very narrow zone and moves from particle surface towards its center, while water is excluding and volume of particle is decreasing.

**Internal diffusion in solution-polysaccharide hydro gel system**

Mechanism of compounds mass transfer in solution-polysaccharide hydro gel system is quite difficult not only by means of biological process but also by behavior and interaction between solution compounds. A basic mechanism which occurs in diffusion process is molecular diffusion. In chemical engineering applications is diffusion flux of compound ($j_i$) in polymers expressed by equation analogical to 1.Fick’s law. The most often used is quasi homogeneous model of particle.

$$j_i = -D_i^* \nabla c_i^*$$  \hspace{1cm} (1)

$D_i^*$ -diffusion coefficient of compound in polymer; can not be considered as binary diffusion coefficient in pure solvent- $D_{ij}$. 

...represent concentration of compound in polymer expressed as ratio of weight of compound and overall volume of solid phase.

The easiest model in reactor engineering is based on concept where particle consists of solid material with channels leading from particle surface towards its center and mass transfer is caused by molecular diffusion.

\[ j_i = -D_{ei} \nabla c_i \]  

(2)

\( D_{ei} \) - effective diffusion coefficient of compound.

\( C_i \) – is real concentration of compound in liquid phase in particle pores.

By comparison of equations (1) and (2) following equation can be obtained

\[ D_{ei} = e_p D^*_i \]  

(3)

\( E_p \) – represents porosity of solid material expressed as ratio of volume of pores (\( V_p \)) to volume of solid phase (\( V_s \)).

Equation (3) is not fully correct, because equation (2) is valid only for molecular diffusion, while in equation (1) are allowed also other mechanisms of diffusion and interaction between compound and polymer.

There for is better write this equation in following form

\[ D_{ei} = K_{ip} D^*_i \]  

(4)

Where \( K_{ip} \) is compound distributing coefficient defined as

\[ K_{ip} = \left( \frac{c^*_i}{c_i} \right)_{rovniava} \]  

(5)

which includes exclusion and distribution effect of gel for each compound.

Distributing coefficient is equal to porosity only if distributing effect is negligible, what we can assume in our case.

Value of distribution coefficient is more accessible parameter which can be obtained from material balance

\[ V_L c_{L0} = V_L c_{L0} + V_S K_{ip} c_{L0} \]  

(6)

unlike porosity which is in ionophylic hydrogels difficult to determine.

\( c_{L0} \)-starting concentration of compound in solution

\( c_{L0} \) - equilibrium concentration of compound on particle surface

\( V_L \) - overall volume of liquid phase.
Determination of effective diffusion coefficient

The most appropriate distribution of methods which are used for effective diffusion coefficient determination is partition in

- Methods with chemical reaction
- Methods without chemical reaction

Effective diffusion coefficient which is determined in steady state system with chemical reaction is not equal to values obtained by methods without chemical reaction. The basic problem of determination of $D_e$ values by methods with chemical reaction is given by correlation of effective diffusion coefficient with kinetics parameters and there for are values of $D_e$ often determined apart (e.g. by sorption method)

The sorption methods differs from method of diffusion cell by transport of a compound which proceed between liquid and solid phase alike of diffusion cell method where the transport take place from one liquid phase to another.

Description of diffusion process is base on material balance of diffusing compound

$$\varepsilon_p \frac{\partial c}{\partial t} = D_e \nabla^2 c$$

(7)

The most commonly used technique for determination of effective diffusion coefficients in hydrogels is diffusion from or to solution with limited volume.

It is assumed that intensity of stirring is satisfactory and so the effect of external diffusion can be neglected.

Material balance of compound, boundary and initial condition can be written as following

$$\varepsilon_p \frac{\partial c}{\partial t} = D_e \left( \frac{\partial c}{\partial r} + \frac{2}{r} \frac{\partial c}{\partial r} \right)$$

(8)

$$t = 0 \quad c = 0$$

(9)

$$r = 0 \quad \frac{\partial c}{\partial r} = 0$$

(10)

$$r = R \quad V_L \frac{\partial c}{\partial t} = -D_e \frac{\partial c}{\partial r} n4\pi R^2$$

(11)

By rearrangement of equation (11)

$$\frac{\partial c}{\partial t} = -\frac{3}{\alpha} \frac{D_e}{R} \frac{\partial c}{\partial r}$$

(12)

Where $t$ – time, $r$-distance from particle centre, $R$-particle radius, $V_L$-volume of liquid phase, $n$-number of particles, $\alpha$ -ratio of liquid phase volume to particles volume.

$$\alpha = \frac{V_L}{V_s} = \frac{V_L}{\frac{4}{3} \pi R^3}$$

(13)

Physical interpretation of equation (12) is simple. Speed of compound decreasing in solution is equal to speed of diffusion of this compound into the particles.
Partial differential equation can be solved by common numerical methods but also can be solved analytically by Laplace transformation. The analytical solution of equation (8) can be than written as

\[
\frac{c_L}{c_{L,\infty}} = 1 + \sum_{n=1}^{\infty} \frac{6\varepsilon_p (\varepsilon_p + \alpha)}{9\varepsilon_p^2 + 9\varepsilon_p\alpha + (\alpha q_n)^2} \exp\left( -\frac{D_e q_n^2 t}{\varepsilon_p R^2} \right)
\] (14)

where \(c_L, c_{L,\infty}\) are concentration in specific time or in equilibrium at the particle surface respectively and \(q_n\) are non zero roots of transcendent equation

\[
tg q_n = \frac{3\varepsilon_p q_n}{3\varepsilon_p + \alpha q_n^2}
\] (15)

From overall compound mass balance (6) and equation (13) can be distribution coefficient determined

\[
K_p = \left( \frac{c_{L,0}}{c_{L,\infty}} - 1 \right) \alpha
\] (16)

When the dependency of concentration on time is measured and the ratio of liquid and solid phase, as well as distribution coefficient (porosity) and particle diameter is also known, the only unknown parameter in equation (14) remains effective diffusion coefficient. Its value can be calculated by one parameter optimization of time-concentration dependency. In this work is used method where by Fibonacci method for each time is one value of \(D_e\) calculated with precision \(1.10^{-14}\) ms\(^{-1}\) and the roots of transcendent equation (15) are calculated by dividing intervals method (METODA POLENIJA INTERVALOV). The representative value of \(D_e\) is than obtained as median of calculated data

**Materials and methods**

**Preparation of spherical Ca-alginate particles**

Ca-alginate particles are prepared by dropping of 1.5% Na-alginate solution into 0.2M CaCl\(_2\) solution (for both solutions distillated water is the solvent) and stirring by magnetic stirrer. When drop is contacted with CaCl\(_2\) solution practically immediately reaction occurs what cause that particles keep their spherical shape. Afterwards particles stay in solution for about 0.5-1 hour so cross linking (gelification) can take place.

The particles are several times washed by distilled water prior the main experiment. During the washing the residual CaCl\(_2\) is removed which could interfere during refractive analysis of substrate concentration.

**Measurement of particle diameter**

Particle diameter is measured by Abbe’s comparator (CZ-Jena, Germany) as average value from 15 values of alginate particles.

**Estimation of substrate concentration**

Substrate concentration (sacharose or glucose) is measured by immersion refractometer (CZ-Jena, Germany) at temperature \(30 \pm 0.1^\circ\)C. The conversion of refractometer values to concentration is based on 2 point calibration from known substrate concentrations.
Equipment description

Equipment for diffusion coefficient determination consists of thermostat, tempered vessel with mechanical stirrer, light source (Na discharge tube) and immersion refractometer.

Apparatus and measurement

Measurement of diffusion coefficients take place in tempered vessel with 5 cm diameter and volume of 150ml equipped by mechanic stirrer. All experiment are proceeded with working volume of liquid phase of 75ml. Samples (0.05ml) are taken by micropipette just after adding of particles (15ml) into stirred and well tempered substrate solution for 30 minutes in different time intervals. Chosen time of experiment is long enough to obtain equilibrium in the system. By this measurement values of concentration dependency on time are obtained and according equation (16) distribution coefficient is calculated.

Aims of the work

1. Measure concentration dependency on time from 2 point calibration curve.
2. Calculate distribution coefficient (porosity) according to equation (16).
3. Measure diameter of spherical alginate particles by Abbe’s comparator.
4. Calculate values of effective diffusion coefficient and determine De by median value. Final De value is obtained as average value from 3 experimental measurements
5. Compare calculated value of effective diffusion coefficient with binary coefficient of measured compound.

Operating procedure

1. Turn on the thermostat for tempering experimental vessel equipped by mechanical stirrer. Set the temperature at 30°C.
2. Turn on Na discharge tube.
3. During temperature conditioning:
   • Measure particle diameter by Abbe’s comparator from 15 random particles.
   • Separate adequate amount of particles from store solution by air pump.
   • Weigh 15g alginate particles for each experiment, because density of these particles is quite similar to density of water we can assume that 15g of particles is equal to 15ml.
   • Prepare 300ml store solution of substrate (glucose or sacharose) with concentration of 100 km$^{-3}$; for each experiment we need 75ml from this store solution.
4. Add measured amount of alginate particles into the tempered stirrer equipped vessel.
5. After obtaining required temperatures in thermostat measure substrate concentration by immerse refractometer which is substrate starting concentration.
6. Well tempered solution pour into the vessel with already inserted particles.
7. Take samples (0.05ml) in different time periods by micropipette for 30min when the experiment is ended. Samples are taken in 0, 20, 40, 60, 90, 120, 150, 190, 240, 300, 360, 420, 480, 600, 900, 1200, 1500 second.
8. Measure refractive indexes of starting substrate concentration (known concentration) and distilled water (zero substrate concentration), from which two point calibration curve is determined.
9. Turn off all equipments, also stop water to the thermostats if was cooling necessary.
10. Wash and dry all used laboratory glass.
Executing of measured date

1. In PC editor create data file (in *.txt format) for each experiment containing following data (keep order of parameters and specified format):

\[ n, \alpha, c_{L,\infty}, r, \varepsilon_p, a, b, \varepsilon, t_i, c_{Li} \]

- \( n \) - number of measurements (integer)
- \( \alpha \) - ratio of volume of liquid and solid phase (real)
- \( c_{L,\infty} \) - equilibrium concentration (kg m\(^{-3}\)) (real)
- \( r \) - particle radius (cm) (real)
- \( \varepsilon_p \) - porosity (real)
- \( a \) - lower value of interval where the solution of \( D_e \) (cm\(^{-2}\)s\(^{-1}\)) is searched (0.)
- \( b \) - upper value of interval where the solution is searched (1.10\(^{10}\))
- \( t_i \) - measured time data (s) (real)
- \( c_{Li} \) - measured concentration interval (kg m\(^{-3}\)) (real)

**Data file example (example.txt)**

```
18, 5., 85.5, 0.154
0.95
0., 1.e-5, 1.e-10
0., 101.4
20., 97.3
... ...
1800., 85.5
```

2. Data files from all 3 experiments in this form will be executed in for this purpose prepared program

3. The results from the program will be process according to higher mentioned procedure.